Annals of Phytomedicine 6(2): 162-168, 2017
DOI: 10.21276/ap.2017.6.2.17; Print ISSN : 2278-9839 and Online ISSN : 2393-9885
Ann. Phytomed., 6(2): 162-168 (2017)

Original article

In vitro acaricidal effects of ethanolic extract and its fractions of *Ageratum conyzoides* L. against common cattle tick, *Rhipicephalus (Boophilus) annulatus*

Suresh Narayanan Nair, Sanis Juliet, Krishnan Kavillimakkil Amithamol, Athalathil Ramankutty Sunil, Adarsh Krishna Thumadath Palayullaparambil, Kanapadinchareveetil Sreelekha, Thirumangalath Meethil Divya, Darsana Udayan, Karapparambu Gopalan Ajith Kumar, Srikanta Ghosh* and Reghu Ravindran

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala-673 576, India

Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala-673 576, India

**Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh-243122, India*

Received July 2, 2017: Revised August 21, 2017: Accepted August 25, 2017: Published online December 30, 2017

Abstract

The *in vitro* acaricidal effect of ethanolic extract and its hexane, chloroform, n-butanol and aqueous fractions were tested for acaricidal activity in fully engorged adult female, *Rhipicephalus (Boophilus) annulatus* based on adult immersion test. The results were compared with standard acaricidal drug deltamethrin. The crude extract showed more acaricidal property compared to the fractions with an LC_{50} of 89.95 mg/ml and LC_{95} 117 mg/ml. Among fractions, the maximum mortality was observed with chloroform fraction with an LC_{50} of 113 mg/ml while the maximum inhibition of fecundity was with the hexane fraction. Hatching of eggs laid by the treated ticks was inhibited by crude extract and its hexane fraction. It may be concluded that the acaricidal effect of *Ageratum conyzoides* L., is produced by more than one component, which may be of non-polar nature.

***Key words*:** *Ageratum conyzoides* L., *Rhipicephlaus (Boophilus) annulatus*, acaricidal activity, deltamethrin

1. Introduction

Parasitic diseases are considered as a major obstacle in the health and performance of animals. Among ectoparasites, ticks are very important and harmful blood sucking external parasites of mammals, birds and reptiles throughout the world. The major losses caused by ticks are due to their ability to transmit protozoal, rickettsial and viral diseases of livestock, which are of great economic importance world-wide (Rajput *et al*., 2006). Moreover, it is estimated that a single *Rhipicephalus (Boophilus) microplus* female tick on an average, is responsible for the loss of 1.18 ± 0.21 g body weight in crossbred cattle (Jonsson, 2006), thus leading to a huge production loss in animal husbandry sector. Usually these ectoparasites are controlled by the use of commercial chemical acaricides available in the market. However, because of the delayed chemical degradation, their residues usually remain in environment which adversely affects the life of living organisms in the natural ecosystem. Another disadvantage with the conventional acaricides is the development of resistance by the ticks against these chemicals. Hence, as an alternative, the interest in development of alternative methods for the control of ticks is dramatically increased in recent years, in accordance with increasing demands for safer animal products and environmental protection (Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007). This has turned the attention of research community for the use of plants or plant products as acaricides. India harbours about 15 per cent out of the 20,000 medicinal plants of the world and the rural population of India largely depends on medicinal plants for their healthcare as well as for their livestock (Anilkumar, 2000). Validation of this knowledge is essential and may pave the way to the discovery of an alternate method and more significantly, to the development of a new drug molecule.

Ageratum conyzoides L. (Goat weed, Family Asteraeceae) is an erect, herbaceous annual, 30 to 80 cm tall plant with stems covered with fine white hairs while leaves are opposite, pubescent with long petioles and include glandular trichomes. The inflorescence contains 30 to 50 pink flowers arranged as a corymb and is self-incompatible (Ming, 1999). Ageratum is derived from Greek word 'ageras' meaning non-ageing while conyzoides from 'konys', a Greek plant *Inula helenium* to which the plant resembles (Kamboj and Saluja, 2008). Previously, the plant's activity against the adult (EL-Kamali, 2009) and larvae (Amelot *et al*., 2003) of storage pest, *Tribolium castaneum* were reported. Besides, antihyperglycaemic (Nyunai *et al*., 2010), fungistatic (Iqbal *et al*., 2004), wound healing (Oladejo *et al*., 2003) and haematopoetic (Ita *et al*., 2007) activities of the plant were also reported.

Rhipicephalus (Boophilus) annulatus is a one host tick under subgenus *Boophilus* within the genus *Rhipicephalus* and occurs in

Author for correspondence: Dr. Suresh Narayanan Nair
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala-673 576, India
E-mail: snn11@rediffmail.com
Tel.: +91-9446919146

greatest abundance in tropical and subtropical regions of the world. This cattle tick not only affects production but also acts as the vector of many protozoal, viral and rickettsial diseases (Wall and Shearer, 1997; Roberts and Janovy, 2005). Even though, *R. (B.) microplus* is the major tick affecting cattle of northern parts of India; *R. (B.) annulatus* is the commonest species found in southern India (Jagannath *et al*., 1979; Koshy *et al*., 1982; Rajamohanan, 1982). The present study evaluates the potential of an ethanolic extract and its hexane, chloroform, n-butanol and water fractions of *A. conyzoides* against *R. (B.) annulatus*, as an acaricide based on adult immersion test.

2. Materials and Methods

All the chemicals used in the extraction and experimentation procedure were procured from M/s Merck India Ltd., Bangalore, India.

The aerial parts of *A. conyzoides* were collected from Vythiri taluk of Wayanad district, Kerala during winter (December) season in year 2009, identified and authenticated by a botanist. The voucher specimen was deposited in the herbarium of Department of Botany, University of Calicut (CALI, Accession No: 6636), Kerala, India. The plant parts were cleaned and dried in shade at room temperature with relative humidity of 70 per cent. The dried plant material was pulverized into a coarse powder using a temperature regulated pulverizer. The powder was extracted with ethanol in a Soxhlet extraction apparatus for (Buchi, Switzerland) eight reflux cycles and the solvents were removed under reduced pressure 86 mbar in rotary vacuum evaporator (Rotavac, M/s Buchi, Switzerland). The crude ethanolic extract was dried at room temperature. The dried crude ethanolic extract was dissolved in methanol to prepare 5%, 6%, 7%, 8%, 9%, and 10% solutions (50, 60, 70, 80, 90 and 100 mg/ml, respectively).

The differential solubility of various components in solvents in an array of ascending polarity was used for separation of fractions from crude ethanolic extract. Ethanolic extract (20 g) was mixed with hexane, a solvent with low polarity, in a separating funnel and soluble fraction collected. The insoluble fraction was serially extracted with chloroform, n-butanol and water, respectively, in the order of ascending polarity. The solvents were removed from the fractions using rotary vacuum evaporator to obtain hexane fraction (HF), chloroform fraction (CF), n-butanol fraction (BF) and water soluble fraction (WF). Different dilutions of each fractions (1.25, 2.5, 5 and 10 mg/ml, respectively) were prepared by dissolving them in methanol and tested for their acaricidal efficacy. The LC_{50} was calculated using graphical method of Miller and Tainter (Finney, 1952).

The fully engorged adult females, *R. (B.) annulatus* were collected from infested animals. They were washed in tap water and dried on an absorbent paper and were used for testing of biological activity. For testing the crude extract, 168 adult engorged female ticks were used, which were divided into eight groups (six treatment group and one control and one standard group). Each group consisted of twenty-four ticks divided into four replicates. Each group was used to estimate the acaricidal effect of respective concentrations. Similarly, for each fraction, 120 adult engorged female ticks were used, dividing them into five groups (four treatment group and one control group), each group structured as above. Each replicate was placed in separate specimen tube and their weights were determined prior to the trial.

The ticks were immersed in 10 ml of the test solution for two minutes and then recovered from the solution, dried with filter paper and incubated at 28°C and 80 per cent relative humidity. Methanol was used as negative control for crude ethanolic extract, chloroform and n-butanol fractions. Tween 5 per cent and water were used as negative control for hexane and water fractions, respectively. Deltamethrin (> 99.9 per cent purity, accustandard, USA) at the dose rate of 0.03 mg/ml was used as positive control. The treated ticks were observed for mortality up to 15 days and for oviposition. The per cent adult mortality and the weight of eggs laid by the treated ticks were recorded in comparison with the control. The eggs were incubated at the same conditions and the per cent hatchability was estimated visually. The index of egg laying and percentage inhibition of fecundity was calculated using the following formulae (1) and (2), respectively (FAO, 2004).

$$\text{Index of egg laying (IE)} = \frac{\text{weight of eggs laid (mg)}}{\text{weight of females (mg)}}$$

$$\text{Percentage inhibition of fecundity (IF)} = [\text{IE (control group)} - \text{IE (treated group)}] \times 100 / \text{IE (control group)}$$

All the data were expressed as mean ± SEM. Groups were compared using one-way ANOVA, and for repeated measurements using SPSS software. Duncan's test was used for post hoc analysis. A value of $p \leq 0.05$ was considered significant.

3. Results

The mean adult tick mortality, percent inhibition of fecundity and hatching percentage of eggs laid by the crude ethanolic extract treated ticks are shown in Table 1.

The crude ethanolic extract of *A. conyzoides* produced per cent adult mortality, which varied from 0 to 62.4957 ± 10.485 at concentrations from 50-100 mg/ml. The probit analysis revealed an LC_{50} as 8.995 mg/ml and LC_{95} of 11.7 mg/ml. The percentage inhibition of fecundity ranged from 15.97 to 58.69 when treated with the crude extract. At concentrations above 80 mg/ml, the total eclosion blocking was observed. For the hexane fraction, the per cent adult mortality ranged from 12.495 ± 4.1650 to 20.8275 ± 4.1675. The LC_{50} for this fraction was 113 mg/ml. The per cent inhibition of fecundity ranged from 17.99 to 46.77 for hexane fraction and 8.52 to 33.38 for chloroform fraction for concentrations ranging from 12.5 mg/ml to 100 mg/ml. Mean adult mortality of chloroform fraction varied from 4.165 ± 2.126 to 45.83 ± 7.978. Though, the n-butanol and aqueous fraction did not have any adulticidal activity, it showed an inhibition of fecundity which ranged from 21.64 to 23.93, while aqueous fraction revealed an inhibition percentage of 11.1 to 16.92. The hexane fraction showed 90-95 per cent of eclosion blocking when treated at higher concentrations (5-10 per cent).

Table 1: Effects of different dilutions of ethanolic extract of *A. conyzoides* against *R. (B.) annulatus*

Sl. No.	Acaricide	Mean ticks weight per replicate ± SEM (g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage inhibition of fecundity (%)	Hatching % (Visual)
1.	Methanol	0.8691 ± 0.0426^{ab}	0 ± 0^{a}	0.3765 ± 0.0220^{d}	0.4365 ± 0.0330^{d}	0	100
2.	Deltamethrin (30 ppm/ 0.03 mg/ml)	0.9653 ± 0.0361^{b}	16.662 ± 6.803^{a}	0.2081 ± 0.0276^{ab}	0.2140 ± 0.0236^{ab}	57.3	0
3.	50 mg/ml	0.8657 ± 0.0288^{ab}	0 ± 0^{a}	0.3166 ± 0.0276^{cd}	0.3668 ± 0.3280^{cd}	15.97	5
4.	60 mg/ml	0.9197 ± 0.0505^{ab}	16.665 ± 9.6215^{a}	0.2940 ± 0.0435^{bcd}	0.3149 ± 0.3442^{bc}	27.86	5
5.	70 mg/ml	0.8457 ± 0.0438^{ab}	16.665 ± 6.8035^{a}	0.2739 ± 0.112^{bcd}	0.3267 ± 0.0233^{bcd}	25.15	5
6.	80 mg/ml	0.8292 ± 0.0251^{a}	16.665 ± 6.8035^{a}	0.2499 ± 0.0255^{abc}	0.3042 ± 0.0390^{bc}	30.31	0
7.	90 mg/ml	0.9533 ± 0.0376^{ab}	41.665 ± 4.8122^{b}	0.2015 ± 0.0346^{ab}	0.2135 ± 0.0384^{ab}	51.09	0
8	100 mg/ml	0.8453 ± 0.0469^{ab}	62.4957 ± 10.485^{c}	0.1569 ± 0.0546^{a}	0.1803 ± 0.0576^{a}	58.69	0

n = 4, Values are mean ± SEM calculated with SPSS and post hoc with Duncan multiple comparison, means bearing different superscripts a, b, c or d ($p \leq 0.05$), indicate significant difference when compared with the control and recommended concentration of deltamethrin

The data obtained when treated with hexane, chloroform, n–butanol and water fractions are given in the Tables 2 to 5 and Graph 1.

Table 2: Effects of different dilutions of hexane fraction of ethanolic extract of *A. conyzoides* against *R. (B.) annulatus*

Sl. No.	Acaricide	Mean ticks weight per replicate ± SEM (g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage inhibition of fecundity (%)	Hatching % (Visual)
1.	5 % tween20	1.0111 ± 0.0354^{ab}	0 ± 0.0000^{a}	0.4636 ± 0.0175^{c}	0.4585 ± 0.0072^{c}	0	100
2.	Deltamethrin (30 ppm/ 0.03 mg/ml)	1.0111 ± 0.0354^{ab}	16.6625 ± 6.8035^{b}	0.2081 ± 0.0277^{a}	0.214 ± 0.0236^{a}	57.3	0
3.	12.5 mg/ml	1.0111 ± 0.0354^{ab}	12.495 ± 4.1650^{ab}	0.3839 ± 0.0140^{b}	0.376 ± 0.0247^{b}	17.99	50
4.	25 mg/ml	1.0111 ± 0.0354^{ab}	12.495 ± 4.1650^{ab}	0.3844 ± 0.0301^{b}	0.3529 ± 0.0357^{b}	23.33	50
5.	50 mg/ml	1.0111 ± 0.0354^{ab}	12.495 ± 4.1650^{ab}	0.365 ± 0.0363^{b}	0.3413 ± 0.0288^{b}	25.56	10
6.	100 mg/ml	1.0111 ± 0.0354^{ab}	20.8275 ± 4.1675^{b}	0.2747 ± 0.0363^{a}	0.244 ± 0.0084^{a}	46.77	5

n = 4, Values are mean ± SEM calculated with SPSS and post hoc with Duncan multiple comparison, means bearing different superscripts a, b, c or d ($p \leq 0.05$), indicate significant difference when compared with the control and recommended concentration of deltamethrin

Table 3: Effects of different dilutions of chloroform fraction of ethanolic extract of *A. conyzoides* against *R. (B.) annulatus*

Sl. No.	Acaricide	Mean ticks weight per replicate ± SEM (g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage inhibition of fecundity (%)	Hatching % (Visual)
1.	Methanol	0.9336 ± 0.0235^{bc}	0 ± 0.0000^{a}	0.4332 ± 0.0173^{b}	0.465 ± 0.0224^{b}	0	100
2.	Deltamethrin (30 ppm/ 0.03 mg/ml)	0.9653 ± 0.0361^{c}	16.6625 ± 6.8035^{ab}	0.2081 ± 0.0277^{a}	0.214 ± 0.0236^{a}	57.3	0
3.	12.5 mg/ml	0.7889 ± 0.0272^{a}	4.165 ± 2.126^{a}	0.3304 ± 0.0511^{ab}	0.4254 ± 0.0770^{b}	8.52	100
4.	25 mg/ml	0.7846 ± 0.0312^{a}	16.6625 ± 6.8035^{ab}	0.3297 ± 0.0648^{ab}	0.4232 ± 0.0875^{b}	8.99	100
5.	50 mg/ml	0.8156 ± 0.0330^{a}	24.98 ± 4.8209^{b}	0.294 ± 0.0362^{a}	0.3621 ± 0.0446^{ab}	22.13	100
6.	100 mg/ml	0.8453 ± 0.0469^{ab}	45.83 ± 7.9780^{c}	0.2559 ± 0.0313^{a}	0.3098 ± 0.0523^{ab}	33.38	100

n = 4, Values are mean ± SEM calculated with SPSS and post hoc with Duncan multiple comparision, means bearing different superscripts a, b, c or d ($p \leq 0.05$), indicate significant difference when compared with the control and recommended concentration of deltamethrin

Table 4: Effects of different dilutions of n-butanol fraction of ethanolic extract of *A. conyzoides* against *R. (B.) annulatus*

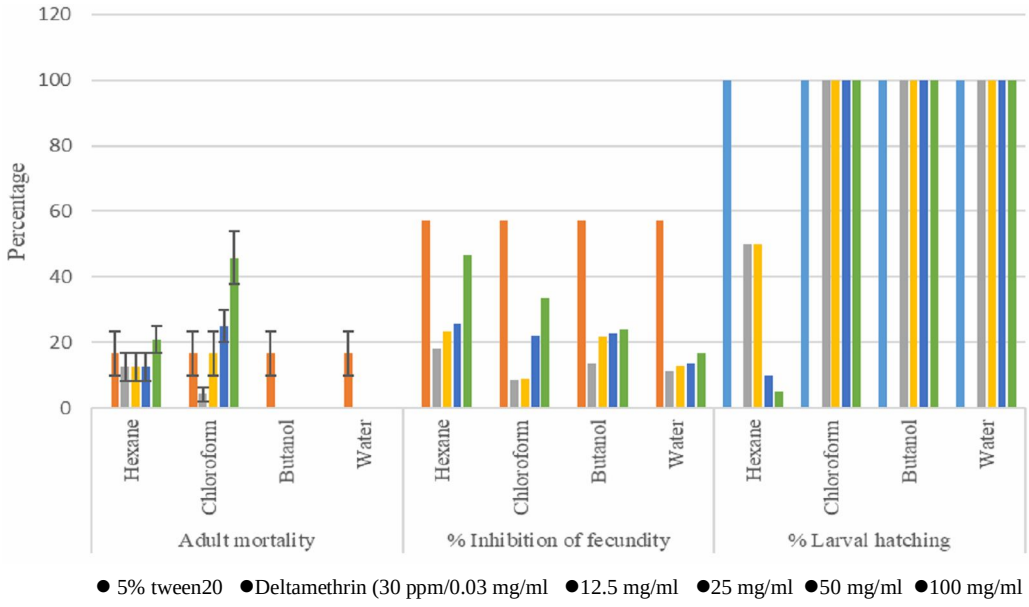
Sl. No.	Acaricide	Mean ticks weight per replicate ± SEM (g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage inhibition of fecundity (%)	Hatching % (Visual)
1.	Methanol	1.0315 ± 0.0415^{ab}	0 ± 0.0^{a}	0.4454 ± 0.0115^{b}	0.433 ± 0.0128^{c}	0	100
2.	Deltamethrin (30 ppm/ 0.03 mg/ml)	0.9653 ± 0.0361^{a}	16.662 ± 6.803^{b}	0.2081 ± 0.0277^{a}	0.214 ± 0.0236^{a}	57.3	0
3.	12.5 mg/ml	1.0644 ± 0.0295^{ab}	0 ± 0.0^{a}	0.357 ± 0.0314^{b}	0.3742 ± 0.0247^{bc}	13.58	100
4.	25 mg/ml	1.149 ± 0.0729^{b}	0 ± 0.0^{a}	0.4311 ± 0.0455^{b}	0.3351 ± 0.0269^{b}	21.64	100
5.	50 mg/ml	1.1504 ± 0.0694^{b}	0 ± 0.0^{a}	0.3927 ± 0.0632^{b}	0.3393 ± 0.0455^{b}	22.61	100
6.	100 mg/ml	1.182 ± 0.0241^{b}	0 ± 0.0^{a}	0.3889 ± 0.0192^{b}	0.3294 ± 0.0173^{b}	23.93	100

n = 4, Values are mean ± SEM calculated with SPSS and post hoc with Duncan multiple comparision, means bearing different superscripts a, b, c or d ($p \leq 0.05$), indicate significant difference when compared with the control and recommended concentration of deltamethrin

Table 5: Effects of different dilutions of water fraction of ethanolic extract of *Ageratum conyzoides* against *R. (B.) annulatus*

Sl. No.	Acaricide	Mean ticks weight per replicate ± SEM (g)	Mean % adult mortality within 15 days ± SEM	Mean eggs mass per replicate ± SEM (g)	Index of fecundity ± SEM	Percentage inhibition of fecundity (%)	Hatching % (Visual)
1.	Water	0.7914 ± 0.0307^{a}	0 ± 0.0^{a}	0.3568 ± 0.0148^{b}	0.4551 ± 0.0373^{b}	0	100
2.	Deltamethrin (30 ppm/ 0.03 mg/ml)	0.9653 ± 0.0361^{b}	16.662 ± 6.803^{b}	0.2081 ± 0.0277^{a}	0.214 ± 0.0236^{a}	57.3	0
3.	12.5 mg/ml	0.802 ± 0.0590^{a}	0 ± 0.0^{a}	0.3143 ± 0.0275^{b}	0.4046 ± 0.0612^{b}	11.1	100
4.	25 mg/ml	0.816 ± 0.0270^{a}	0 ± 0.0^{a}	0.321 ± 0.0223^{b}	0.3971 ± 0.0388^{b}	12.74	100
5.	50 mg/ml	0.8484 ± 0.0195^{a}	0 ± 0.0^{a}	0.3353 ± 0.0256^{b}	0.3941 ± 0.0246^{b}	13.4	100
6.	100 mg/ml	0.8348 ± 0.0279^{a}	0 ± 0.0^{a}	0.315 ± 0.0092^{b}	0.3781 ± 0.0117^{b}	16.92	100

n = 4, Values are mean ± SEM calculated with SPSS and post hoc with Duncan multiple comparision, means bearing different superscripts a, b, c or d ($p \leq 0.05$), indicate significant difference when compared with the control and recommended concentration of deltamethrin



Graph 1: Effects of various fraction of ethanolic extract of *A. conyzoides* against *R.*(*B.*) *annulatus*

The crude ethanolic extract of *A. conyzoides* produced per cent adult mortality, which varied from 0 to 62.4957 ± 10.485 at concentrations from 50-100 mg/ml. The probit analysis revealed an LC_{50} as 8.995 mg/ml and LC_{95} of 11.7 mg/ml. The percentage inhibition of fecundity ranged from 15.97 to 58.69 when treated with the crude extract. At concentrations above 80 mg/ml, the total eclosion blocking was observed. For the hexane fraction, the per cent adult mortality ranged from 12.495 ± 4.1650 to 20.8275 ± 4.1675. The LC_{50} for this fraction was 113 mg/ml. The per cent inhibition of fecundity ranged from 17.99 to 46.77 for hexane fraction and 8.52 to 33.38 for chloroform fraction for concentrations ranging from 12.5 mg/ml to 100 mg/ml. Mean adult mortality of chloroform fraction varied from 4.165 ± 2.126 to 45.83 ± 7.978. Though the n-butanol and aqueous fraction did not have any adulticidal activity, it showed an inhibition of fecundity which ranged from 21.64 to 23.93, while aqueous fraction revealed an inhibition percentage of 11.1 to 16.92. The hexane fraction showed 90-95 per cent of eclosion blocking when treated at higher concentrations (5-10 per cent).

4. Discussion

The crude ethanolic extract of *A. conyzoides* produced per cent adult mortality, which varied from 0 to 62.4957 ± 10.485 at concentrations from 50-100 mg/ml. Parveen *et al*. (2014) reported a low adult mortality of 10 ± 5.77% in *R. microplus* ticks at 10% (100 mg/ml) concentration of ethanolic extracts with DMSO and distilled water as solvents. However, Ajith Kumar *et al*. (2016) reported high mortality at lower concentration with an LC_{50} of 3.94% and LC_{90} of 8.91%. High mortality in the present study may be either due to the variation in the activity of plants collected from different regions/ season or due to the solvent used for preparation of stock and working solution. Significantly high level of variation in the antitick activity of the plant extract was noted when collected from different states of India (Ajith Kumar *et al*., 2016). At concentrations of 90 and 100 mg/ml, statistically significant increase in mortality was observed when compared to deltamethrin. The probit analysis revealed an LC_{50} as 8.995 mg/ml and LC_{95} of 11.7 mg/ml. The standard acaricidal chemical compound deltamethrin showed an adult mortality of 16.7 per cent at the dose rate of 0.03 mg /ml.

Similarly, percentage inhibition of fecundity ranged from 15.97 to 58.69 when treated with the crude extract. Deltamethrin at 0.03 mg / ml concentration produced 57.3 per cent inhibition of fecundity. Statistical similarity is observed with 90 mg/ml concentration, while an increased effect is noted at 100 mg/ml when compared to deltamethrin. The per cent larval hatching was very low (5%) even at lower concentration of crude extract while at concentrations above 8 per cent, the total eclosion blocking was observed.

For the hexane fraction, the per cent adult mortality ranged from 12.495 ± 4.1650 to 20.8275 ± 4.1675 while chloroform fraction revealed a per cent adult mortality range between 4.165 ± 4.1650 and 45.83 ± 7.9780, with a calculated LC_{50} of 113 mg/ml. Chloroform fraction showed significantly higher mortality at 50 mg/ml and 100 mg/mL when compared to deltamethrin, whereas hexane fraction showed significance at 100 mg/ml only. Hence, chloroform extract is the better acaricidal fraction among the fractions. Both the fractions showed lesser adult mortality compared to the crude extract. There was no adult tick mortality in n-butanol and aqueous fractions even at the highest concentration tested. So, it may be inferred that, the active ingredient or ingredients that are responsible for adult tick mortality has more affinity for chloroform than hexane, indicating a lower but not least polarity of the compound.

The per cent inhibition of fecundity ranged from 17.99 to 46.78 for hexane fraction and 8.52 to 33.38 for chloroform fraction for concentrations ranging from 12.5 mg/ml to 100 mg/ml. Unlike the adult mortality, the values of per cent inhibition of fecundity were higher than that of crude extract, indicating that the chemical molecules responsible were being concentrated in non-polar solvents. The n-butanol fraction showed an inhibition of fecundity which ranged from 21.64 to 23.93, while aqueous fraction revealed an inhibition percentage of 11.1 to 16.92. Hence, it can be speculated that either there are two or more active ingredients in the crude extract responsible for inhibition of fecundity or if it is a single compound, it is having a slight polarity. When the polarity of the solvent was increased, there was reduction in inhibition of fecundity. The higher percentage of eclosion blocking effect in hexane may indicate predominant non-polar nature of those compounds.

The hatching of eggs laid by crude ethanolic extract treated ticks was completely blocked at concentrations ranging from 8-10 per cent. However, 90-95 per cent of eclosion blocking was observed in ticks treated at higher concentrations (5-10 per cent) of the hexane fraction. All other fractions did not affect hatching of eggs laid by treated ticks. Hence, it may be assumed that the agent/agents that cause eclosion blocking effect may be slightly polar or non-polar in nature.

Previous reports on phytochemical analysis of the plant revealed that the essential oil contains a benzofuran derivative compound known as chromenes. The two predominant chromenes that are present in the plant are 7-methoxyl-2, 2-dimethylchromene (Prococene I) and 6, 7 - dimethoxy- 2, 2- dimethylchromene (precocene II) (Kamboj and Saluja, 2008). Adebayo *et al*. (2010) reported that precocene II was the main constituent isolated from *A. conyzoides*. Exposure of precocene II to young insects leads to precocious metamorphosis, thereby development of unviable or moribund miniature adult. Exposure of adult female of several insect species to the same compound causes sterility by preventing normal vitellogenic development of oocytes. The compound also showed antijuvenile hormone activity in insects (Pratt and Bowers, 1977). Booth *et al*. (1986) demonstrated that the treatment of adult *B. microplus* with precocene II resulted in absence of water proofing waxy layer of eggs due to destruction of glandular cells of gene's organ. Ribeiro *et al*. (2008) attributed the acaricidal effects of *C. serrata* to precocene II. The effects of precocene II against ticks are reduction of oviposition (Pound and Oliver, 1979; Connat, 1988; Taylor *et al*., 1992) and prevention in development and reproduction (Gaber *et al*., 1983; Khalil *et al*., 1983).

Another chemical compound coumarin, present in the plant extract was also reported to have an insecticidal (Khan *et al*., 2002) and insect growth regulating and antifeedant property (Syamala *et al*., 2010). The inhibition of fecundity observed in the present experiment could be attributed to this compound. Chromenes and coumarines are more soluble in non-polar solvents. The higher per cent inhibition of fecundity observed with the hexane fraction could be due to the enrichment of fractions with these compounds when compared to the crude extract. Also, Calle *et al*. (1991) reported that insecticidal activity of the plant, *A. conyzoides* resides with the petroleum ether extract, another non- polar solvent. Other bioactive molecules isolated from *A. conyzoides* were pyrrolizidine alkaloid compounds like

lycopsamine and echinatine and flavonoids like ageconyflavone A, B and C, eupalestine, quercetin and kaempferol. Pyrrolizidine alkaloids are secondary metabolites of certain plants which are produced by plants against herbivorous insects. Besides, certain triterpenoids and sterols were also reported in the plant (Kamboj and Saluja, 2008). The essential oil of the plant was reported to contain 12-6-methyl heptadecanoic acid (Sayeda *et al*., 2009).

Hence, at least four possible chemical compounds can act synergistically producing acaricidal effects, namely; precocenes, coumarines, fatty acid derivatives and pyrrolizidine alkaloids. Ukwe *et al.* (2010) reported that the hexane fraction of *A. conyzoides* contained flavonoids, fats and oils, alkaloids and resins, whereas the methanolic extract contained saponins and tannins in addition to these glycosides. The chloroform fraction showed flavonoids, and alkaloids (less compared to hexane fraction). This may also possibly explain the reason for enhanced activity of hexane and chloroform fractions compared to the n-butanol fraction.

Even though, insecticidal activity of *A. conyzoides* is well documented in insects and other species of animals, the present study is the first of its kinds against ticks revealing its adulticidal, fecundity inhibition and eclosion blocking activities.

5. Conclusion

The crude extract of *A. conyzoides* showed more acaricidal property compared to the fractions with an LC_{50} of 89.95 mg/ml and LC_{95} 117 mg/ml. Among fractions, the maximum mortality was observed with chloroform fraction with an LC_{50} of 113 mg/ml while the maximum inhibition of fecundity was with the hexane fraction. Hatching of eggs laid by the treated ticks was inhibited by crude extract and its hexane fraction. It may be concluded that the acaricidal effect of *A. conyzoides* is produced by more than one component, which may be of non-polar nature. Even though, insecticidal activity of *A. conyzoides* is well documented in insects and other species of animals, the present study forms the first of its kind against ticks revealing its adulticidal, fecundity inhibition and eclosion blocking activities.

Acknowledgements

Financial supports from Indian Council of Agricultural Research (ICAR) through research projects (NAIP/Comp-4/C2066/2007–08, NFBSFARA/BSA-4004/2013-14, NASF/ABA-6015/2016-17, No. 7(2)/ 2011 EPD), Kerala State Council for Science, Technology and Environment (010-14/SARD/13/CSTE dt 27-11-2013), National Bank for Agriculture and Rural Development G.O.(Rt)No.100/12/ AD RIDF XVI KERALA) and ICAR EFC Centre for Ethnopharmacology are thankfully acknowledged.

Conflict of interest

We declare that we have no conflict of interest.

References

Adebayo, A.H.; Zeng, G.Z.; Zhang, Y. M.; Ji, C. J.; Akidahunsi, A. A. and Tan, N. H. (2010). Toxicological evaluation of precocene II isolated from *Ageratum conyzoides* L. (Astera ceae) in Sprague Dawley rats. Afr. J. Biotechnol., **9**(20):2938-2944.

Ajith Kumar, K.G.; Tayade, A.B.; Kumar, R.; Gupta, S.; Sharma, A.K.; Nagar, G.; Tewari, S.S.; Kumar, B.; Rawat, A.K.S.; Srivastava, S. and Kumar, S. (2016). Chemoprofiling and bioassay of phytoextracts from *A. geratum* conyzoides for acaricidal properties against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) infesting cattle and buffaloes in India. Ticks Tick Borne Dis., **7**(2):342-349.

Amelot, M.E.A.; Avendaño, M.; Aubert, L. and Avila, J.L. (2003). Repellency and feeding deterrence activity of *Ageratum conyzoides* against the stored grain pests *Tribolium castaneum* and *Sitophilus oryzae*. Active plant parts and composition. Ciencia., **11**(1):61-76.

Anilkumar, M. (2010). Ethnomedical plants as anti-inflammatory and analgesic agent. Ethnomedicine: A source of complimentary Therapeutics, pp:267-283.

Booth, T.F.; Beadle, D.J. and Hart, R.J. (1986). The effects of precocene treatment on egg wax production in Gene's organ and egg viability in the cattle tick *Boophilus microplus* (*Acarina ixodidae*): An ultrastructural study. Exp. Appl. Acarol., **2**(2):187-198.

Calle, J.; Rivera, A.; Luis, J.G; Aguiar, Z.E.; Niemeyer, H.M. and Nathan P.J. (1991). Insecticidal activity of petroleum ether extract of *Ageratum conyzoides*. Revista Colombiana de Quimica., **19**(1):91-96.

Connat, J.L. (1988). Effects of different anti-juvenile hormone agents on the fecundity of the female cattle tick *Boophilus microplus*. Pesticide Biochem. Physiol., **30**(1):28-34.

EL-Kamali. (2009). Effect of certain medicinal plants extracts against storage pest, *Tribolium castaneum* Herbst. Am-Eurasian J. Sustain Agric., **3**(2):139-142.

FAO, (2004). Guidelines resistance management and integrated parasite control in ruminants. Rome.

Finney, D.J. (1952). Probit analysis, Cambridge University Press, Cambridge, England.

Gaber, S.H.A.; Khalil, G.M.; Sonenshine, D.E. and Moez, M.A. (1983). Precocene-2 effects on the camel tick *Hyalomma dromedary*. I. Adult responses. J. Med. Entomol., **20**:534-540.

Iqbal, M.C.M.; Jayasinghe, U.L.B.; Herath, H.M.T.B.; Wijesekara, K.B. and Fujimoto, Y. (2004). A fungistatic chromene from *Ageratum conyzoides*. Phytoparasitica, **32**(2):119-126.

Ita, S.O.; Etim, O.E.; Ben, E.E. and Ekpo, O.F. (2007). Haematopoietic properties of ethanolic leaf extract of *Ageratum conyzoides* (Goatweed) in albino rats. Niger. Physiol. Sci., **22**(1-2):83-87.

Jagannath, M.S.; Muraleedharan, K. and Hiregoudar, L.S. (1979). Prevalence of ixodid ticks of cattle at Bangalore. Indian J. Anim. Sci., **49**:890-894.

Jonsson, N.N. (2006). The productivity effects of cattle tick (*Boophilus microplus*) infestation on cattle, with particular reference to *Bos indicus* cattle and their crosses. Vet. Parasitol., **137**(1-2):1-10.

Kamboj, A. and Saluja, A.K. (2008). *Ageratum conyzoides* L: A review on its phytochemical and pharmacological profile. Int. J. Green Pharm., **2**(2):59-68.

Khalil, G.M.; Gaber, S.H.A.; Sonenshine, D.E. and Gad, S.M. (1983). Precocene-2 effects on the camel tick *Hyalomma dromedari*.2. Larval and nymphal responses. J. Med. Entomol., **20**(5):541-550.

Khan, K. M.; Saify, Z.S.; Hayat, S.; Khan, M.Z.; Noor, F.; Makhmoor, T.; Choudhary, M.I.; Zia-Ullah and Perveen, S. (2002). Synthesis, antioxidant and insecticidal activities of some coumarine derivatives. J. Chem. Soc. Pak., **24**(3):226-231.

Koshy, T.J.; Rajavelu, G. and Lalitha C.M. (1982). Ecology and bionomics of boophilids of Tamil Nadu. Cheiron., **11**(1):25-30.

Ming, L.C. (1999). *Ageratum conyzoides*: A tropical source of medicinal and agricultural products perspectives on new crops and new uses. J. Janick (ed.), ASHS Press, Alexandria, VA.

Nyunai, N.; Manguelle-Dicoum, A.; Njifutie, N.; Abdennebi, E. H. and Gerard, C. (2010). Antihyperglycaemic effect of *Ageratum conyzoides* L in normoglycaemic and diabetic male wistar rats. Int. J. Pharm. Biomed. Res., **4**:38-42.

Oladejo, O.W.; Imosemi, I.O.; Osuagwu, F.C.; Oluwadara, O.O.; Aiku, A.; Adewoyin, O.; Ekpo, O. E.; Oyedele, O.O. and Akang, E.E.U. (2003). Enhancement of cutaneous wound healing by methanolic extracts of *Ageratum conyzoides* in the wistar rat. Afr. J. Biomed. Res., **6**(1):27-31.

Parveen, S.; Godara, R.; Katoch, R.; Yadav, A.; Verma, P.K.; Katoch, M. and Singh, N.K. (2014). *In vitro* evaluation of ethanolic extracts of *Ageratum conyzoides* and *Artemisia absinthium* against cattle tick, *Rhipicephalus microplus*. The Scientific World Journal, http://dx.doi.org/10.1155/2014/858973.

Pirali-Kheirabadi, K.H. and Razzaghi-Abyaneh, M. (2007). Biological activitis of chamomile (*Matricaria chamomile*) flowers extract against the survival and egg laying of the cattle fever tick (*Acari ixodidae*). J. Zhejiang Univ. Sci. B., **8**(9):693-696.

Pound, J.M. and Oliver, Jr. J.H. (1979). Juvenile hormone: Evidence of the role in the reproduction of ticks. Science, **206**(4416):355-357.

Pratt, G.E. and Bowers, W.S. (1977). Precocene II inhibits juvenile hormone biosynthesis by cockroach *Corpora allata in vitro*. Nature, **265**(5594): 548-550.

Rajamohanan, K. (1982). Identification of vector for babesiosis of cattle in Kerala. Proceedings of all India symposium of vectors and vectors and vector borne diseases, Trivandrum, Kerala, pp:125-128.

Rajput, Z.I.; Hu, S.; Chen, W.; Arijo, A.G. and Xiao, C. (2006). Importance of ticks and their chemical and immunological control in livestock. J. Zhejiang Univ. Sci. B., **7**(11):912-921.

Roberts, L.S., and Janovy, J. (2005). Foundation of Parasitology (7thEd.) McGraw Hill. pp:644-646.

Ribeiro, V.L.S.; Avancini, C.; Goncalves, K.; Togio, E. and von Poser, G. (2008). Acaricidal activity of *Calea serrata* (Asteraceae) on *Boophilus microplus* and *Rhipicephalus sanguineus*. Vet. Parasitol., **151**(2-4): 351-354.

Sayeda, F.F.; Torkey, H.M. and Abou-Yousef (2009). Natural extracts and their chemical constituents in relation to toxicity against Whitefly (Bemisia *tabaci*) and Aphid (*Aphis craccivora*). Aust. J. Basic Appl. Sci., **3**(4):3217-3223.

Syamala, B.S.; Ananthalakshmi, P.V. and Tyagaraju, V.J. (2010). Synthesis, antifeedant and insect growth regulating activity of N-(2-nitro) benzylidine-3-animo coumarine and their physico-chemical characterization. Int. J. Pharm. Pharm. Sci., **2**(Suppl 4):150-152.

Taylor, D.; Chinzei,Y.; Miura, K. and Ando, K. (1992). Effects of precocenes on the vitellogenesis in the adult female tick *Ornithodoros moubata* (Acari: Argasidae). Exp. Appl. Acarol., **14**(2):123-136.

Ukwe, V.C.; Epueke, E.A.; Ekwunife, O.I.; Okoye T.C.; Akudor, G.C. and Ubaka, C.M. (2010). Antimalarial activity of aqueous extract and fractions of leaves of *Ageratum conyzoides* in mice infected with *Plasmodium berghei*. Int. J. Pharm. Sci., **2**(1):33-38.

Wall, R. and Shearer, D. (1997). Veterinary Entomology. Chappman and Hall. pp:97-140.